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1. Kwak et al., Gene, 1996. Vol. 173(2), 257-259
2. Giunta et al., Annals of the New York Academy of Sciences. 1992, 673, 342-349.
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Bao-Thuy Nguyen
(703) 308-4243
CM 1, Room 7E05

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Cloning and characterization of cDNAs coding for heavy and light chains of a monoclonal antibody (MabA34) specific for human plasma apolipoprotein A-I

(Recombinant DNA; immunoglobulin Fab; Apo A-I; polymerase chain reaction; high-density lipoproteins; HDL chclesterol; DNA sequencing)

Ju-Won Kwak[a], Dong-Ik Lee[a], Byung-Kwon Choi[b], Won-Kyung Cho[a], Sang-Han Lee[c], Yong-Bok Park[d] and Moon Hi Han[a]

[a]*Korea Research Institute of Bioscience and Biotechnology, KIST, Daeduck Science Town, Taejon 305-600, South Korea;* [b]*Sam-Il Pharmaceutical Co. Ltd., Banghae-Dong, Seocho-Ku, Seoul 137-060, South Korea;* [c]*Biochemistry Laboratory, College of Medicine, Soonchunhyang University, Chonan 330-020, South Korea; and* [d]*Department of Genetic Engineering, Kyungpook National University, Taegu 702-701, South Korea*

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SUMMARY

We have determined the nucleotide (nt) sequences encoding the heavy (H)- and light (L)-chains of the Fab fragment of a murine monoclonal antibody, MabA34 (γ1, κ), which is specific for human plasma apolipoprotein A-I of high-density lipoproteins. The variable (V) regions of the H- and L-chains were revealed to be members of mouse H-chain subgroup II(A) and κ L-chain subgroup II, respectively. A few unusual amino acids in the V region of the H-chain, and nt residues probably introduced by somatic mutations from germline genes were also identified.

INTRODUCTION

Plasma apolipoprotein (Apo) A-I of high-density lipoproteins (HDL) has received considerable clinical attention because the plasma concentrations have an inverse relationship with the incidence of coronary artery disease (Avogaro et al., 1979; Naito, 1986; Bachorik and Kwiterovich, 1988). Apo A-I is a major protein component of HDL which is mainly responsible for the reverse cholesterol transport from peripheral tissues to the liver. Following the recognition of an inverse correlation between plasma HDL and the incidence of coronary artery disease, Apo A-I has become the subject for numerous immunochemical studies involving both polyclonal and monoclonal Ab. Here, we report the cloning and characterization of cDNAs encoding the heavy (H)- and light (L)-chains for the Fab fragment of a mAb (MabA34) which specifically recognizes and binds to human plasma Apo A-I of HDL.

Correspondence to: Dr. J.-W. Kwak, Protein Engineering Research Group, Korea Research Institute of Bioscience and Biotechnology, KIST, P.O. Box 115, Yuseong, Taejon 305-600, South Korea. Tel. (82-42) 860-4125; Fax (82-42) 860-4594; e-mail: jwkwak@geri4680.geri.re.kr

Abbreviations: aa, amino acid(s); Ab, antibody(ies); Apo, apolipoprotein(s); bp, base pair(s); C, constant; cDNA, complementary DNA; CDR, complementarity determining region(s); C_H1, H-chain C region 1; C_κ, κ L-chain C region; D, diversity; Fab, antigen-binding Ab fragment; FR, framework region(s); H, heavy; HDL, high-density lipoprotein(s); J_H, H-chain joining segment; J_κ, κ L-chain joining segment; L, light; mAb, monoclonal Ab; nt, nucleotide(s); oligo, oligodeoxyribonucleotide; PC', polymerase chain reaction; V, variable; V_H, H-chain V region; V_L, L-chain V region.

EXPERIMENTAL AND DISCUSSION

(a) Cloning of MabA34-specific heavy and light chain cDNAs

The fusion of mouse Balb/c spleen cells and Sp2/O-Ag-14 myeloma cells has produced the hybridoma cell

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line H-MabA34 secreting MabA34 (γ1, κ). In order to clone the cDNAs coding for the Fab fragment of the mAb, the N-terminal aa sequences of the H- and L-chains were determined, and the oligos specific for the N-terminal aa sequences and constant region genes were synthesized with restriction enzyme sites on the ends. The oligos were used as primers for the reverse transcription of H-MabA34 cytoplasmic RNA followed by PCR. The resulting 640-bp and 660-bp DNA fragments containing the H- and L-chain Fab regions were cloned into pT7Blue and pBluescript to yield pA34HT7 and pA34LBlue, respectively.

(b) Sequence analyses

The nt sequences determined and deduced aa sequences of the H- and L-chains for Fab fragment are shown in Fig. 1. The sequence analysis of the H-chain-specific cDNA revealed that the V region of the H-chain belongs to the mouse H-chain subgroup II(A) of Kabat et al. (1991). The complementarity determining region (CDR) 1, 2 and 3 were positioned at aa 31–35, 50–65, 99–101, respectively. Three unusual residues in FR 1 of the H-chain V region were identified, i.e., Val[5], Glu[6] and Asp[26]. Otherwise, all the invariant aa for the murine H-chain subgroup II(A) were conserved (Harris and Bajorath, 1995). A total of five Cys in the H-chain Fab region and five Cys in the L-chain were found, which would be involved in intra- or inter-chain disulfide bonding necessary to form the Fab immunoglobulin structure. At the end of the H-chain V region (aa 100–112 in Fig. 1), J_H2 element without any nt mismatch was identified. In the CDR3 of the H-chain, a unique D region comprised of only one aa (Pro^{99}) was also identified. Comparisons with germline V genes have assigned the V region of H-chain to 19.1.2, a VhJ558 family (Akolkar et al., 1987), and suggested that the V region had undergone somatic mutations characteristic of an antigen-driven immune response.

The sequence analysis of L-chain-specific cDNA of MabA34 revealed that the V region of the L-chain is a member of mouse κ chain subgroup II (Fig. 2). All the invariant aa for the κ chain were conserved with no exceptions. The CDR 1, 2, and 3 were positioned at aa 24–39, 55–61, 94–102, respectively. At the end of L-chain V region (aa 100–111 in Fig. 2), $J_κ1$ element with 1 nt mismatch (CGG instead of TGG for Arg^{100}) was identified. Comparisons with germline V genes allowed to determine K1A5 (Corbet et al., 1987) as a likely germline progenitor of the V domain of the L-chain.

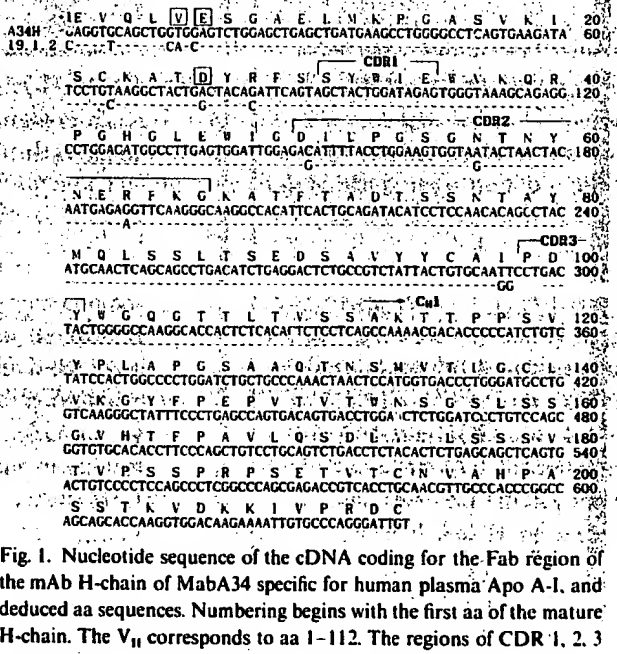

Fig. 1. Nucleotide sequence of the cDNA coding for the Fab region of the mAb H-chain of MabA34 specific for human plasma Apo A-I, and deduced aa sequences. Numbering begins with the first aa of the mature H-chain. The V_H corresponds to aa 1–112. The regions of CDR 1, 2, 3 are blocked. Unusual aa compared with conserved sequences of mouse H-chain subgroup II(A) (Kabat et al., 1991; Harris and Bajorath, 1995) are boxed. The progenitory, mouse germline V gene sequence of 19.1.2 (Akolkar et al., 1987) was aligned with the sequence to show the nt residues probably introduced by somatic mutations. The dashes denote identity. The sequence is available from GenBank database under accession No. U29146. Methods: The PCR product subcloned into pT7Blue (Novagen, Madison, WI, USA) was fully sequenced on both orientations by the dideoxy chain-termination method (Sanger et al., 1977) using M13 universal primer or specific internal primers synthesized, and Sequenase kit (Version 2.0, US Biochemical, Cleveland, OH, USA).

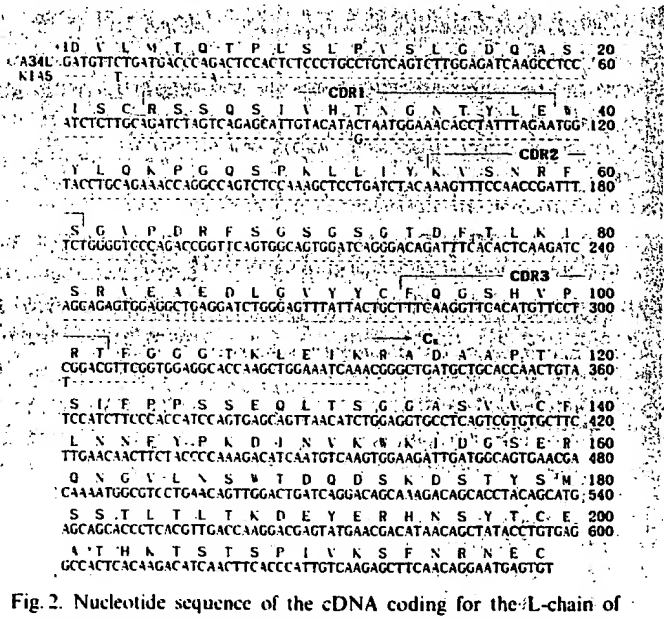

Fig. 2. Nucleotide sequence of the cDNA coding for the L-chain of monoclonal antibody MabA34 specific for human plasma Apo A-I, and deduced aa sequence. The V_L and C_L correspond to aa 1–112 and aa 113–219, respectively. The regions of CDR 1, 2, 3 are blocked. The progenitory, mouse germline V gene sequence of K1A5 (Corbet et al., 1987) was aligned with the sequence to show the nt residues probably introduced by somatic mutations. The dashes denote identity. The sequence is available from GenBank database under accession No. U29147. Methods: The PCR product subcloned into pBluescript (Stratagene, La Jolla, CA, USA) was fully sequenced on both orientations.

The sequence analyses suggest that the H- and L-chain-specific cDNAs are functional. Comparison of nt sequences of the H- and L-chain V region with the available immunoglobulin genes listed in GenBank database showed that the sequences have not been previously reported. The cDNAs will be used for expression and serum-free production in *Escherichia coli* of the recombinant Ab, which is valuable for clinical diagnostics as well as for numerous immunochemical studies of human plasma Apo A-I.

(c) Conclusions

We have cloned and determined the nt sequences of the heavy- and light-chain-specific mAb-encoding cDNAs of murine MabA34 (γ1, κ) specific for human plasma Apo A-I of HDL. The sequence analyses revealed that both cDNAs are functional, and the variable regions of the heavy and light chains are members of mouse H-chain subgroup II(A) and κ L-chain subgroup II, respectively. These sequences have not been previously reported.

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